ADULT HIPPOCAMPAL NEUROGENESIS AFTER EXPERIMENTAL STROKE: A MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION

Dissertation

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2 Abbreviations

ABGCs	Adult-born granule cells
AP	Action potential
BrdU	5-bromo-2'-deoxyuridine
DAB	3,3'-diaminobenzidine
DCX	Doublecortin
(s/m)EPSC	(spontaneous/miniature) Excitatory postsynaptic currents
GABA	γ-aminobutyric acid
g.c.l.	Granule cell layer
GFAP	Glial fibrillary acidic protein
KCC2	Potassium chloride cotransporter 2
LTP	Long-term potentiation
MCAO	Middle cerebral artery occlusion
NKCC1	Sodium potassium chloride cotransporter 1
NSPC	Neural stem/precursor cells
MoCA	Montreal cognitive assessment test
MWM	Morris water maze
PFA	Paraformaldehyde
PP	Perforant path
PSD	Poststroke depression
PTX	Picrotoxin
RFP	Red-fluorescent protein
SGZ	Subgranular zone
Shh	Sonic hedgehog
SVZ	Subventricular zone
TTX	Tetrodotoxin

3 Abstract

Long-term neuropsychiatric sequelae of stroke include post-stroke cognitive impairment and post-stroke depression, as well as epilepsy. The pathophysiology of these conditions is not sufficiently understood, but they may develop even as the infarct core often does not directly affect relevant neurofunctional centers. This suggests a remote or global mode of action of focal/regional ischemic events, e.g. through metaplasticity, leading to neurocognitive impairments. A unique form of brain plasticity is represented by adult neurogenesis, a process common in many species including mammals, as wells as in humans and other primates. The process of adult neurogenesis is affected by multiple physiological and environmental factors but also by disease. Stroke robustly stimulates adult neurogenesis in the hippocampal dentate gyrus. It is, however, currently unknown if this process induces compensatory, beneficial effects or if it is detrimental for the network function. Morphological and behavioral studies have reported aberrant neurogenesis and impaired hippocampal-dependent memory following stroke. However, the functional maturation and network integration of post-stroke adult-born granule cells (ABGCs) has not been investigated.

We used patch-clamp electrophysiology in acute hippocampal slices to characterize immature, doublecortin positive (DCX+) ABGCs two weeks following a stroke or sham operation. Immature ABGCs were identified in a transgenic mouse line which expressed the red fluorescent protein (RFP) DsRed under control of the *DCX* promoter.

We found that following stroke electrophysiological parameters of maturation, including membrane resting potential, input resistance, cell capacitance and membrane time constant were shifted towards a more mature phenotype in DCX+ ABGCs. The intrinsic excitability was also increased after stroke, as more DCX+ ABGCs were able to generate fast-rising action potentials. Morphological maturation after stroke was in keeping with the functional development of ABGCs. Excitatory synaptic activity as measured by spontaneous or miniature excitatory postsynaptic currents (sEPSC and mEPSC) was increased. Furthermore, we used the general linear model to reveal uncoupled developmental dynamics of intrinsic and network excitability in DCX+ ABGCs after stroke. This resulted in young, intrinsically hyperexcitable ABGCs receiving disproportionately large glutamatergic inputs. Therefore, our results point not only to an accelerated intrinsic and synaptic excitability maturation following stroke, but also to an uncoupled development of these parameters, which may have detrimental effects on network function and cognition. The resulting pathological

hyperexcitability in the subgroup of ABGCs in the hippocampus may contribute to mnestic deficits and increased susceptibility for epileptic seizures following stroke.

4 Zusammenfassung

Gedächtnisstörung, Depression und Epilepsie sind einige der möglichen Langzeitfolgen von Schlaganfällen. Die Pathophysiologie dieser Syndrome ist unzureichend verstanden. Diese Langzeitfolgen können auch dann auftreten, wenn der Infarktkern in einem hierfür nicht relevanten funktionellen Zentrum gelegen ist. Dies weist auf eine Fernwirkung oder einen globalen Prozess der fokalen/regionalen Ischämien hin, die die neurokognitiven Defizite vermitteln, bspw. durch Metaplastizität. Die adulte Neurogenese ist eine besondere Form der zerebralen Plastizität und ist in vielen Spezies verbreitet, einschließlich des Menschen und anderer Primaten. Die adulte Neurogenese wird von unterschiedlichen physiologischen und Umgebungsfaktoren sowie von Krankheitsprozessen beeinflusst. Der Schlaganfall verursacht eine ausgeprägte Stimulation der adulten Neurogenese im hippocampalen Gyrus dentatus. Aktuell ist aber nicht bekannt, ob dieser Prozess kompensatorische und nützliche Effekte bewirkt oder er sogar nachteilig für die hippocampale Netzwerkfunktion ist. Morphologische und Verhaltensstudien haben eine aberrante Neurogenese und ein gestörtes Hippocampus-abhängiges Langzeitgedächtnis nachgewiesen. Bislang wurden aber die funktionelle Reifung und die Netzwerkintegration neuer gebildeter Nervenzellen nach einem Schlaganfall nicht untersucht.

Mithilfe der Patch-Clamp-Technik wurden zwei Wochen nach einem experimentellen Schlaganfall oder einer Sham-Operation unreife, Doublecortin-positive (DCX+) neugebildete Neurone (adult-born granule cells, ABGCs) in akuten hippocampalen Hirnschnitten charakterisiert. Unreife ABGCs wurden in einer transgenen Tierlinie, in der das rot fluoreszierende Protein DsRed unter Kontrolle des *DCX* Promotors exprimiert wird, identifiziert.

Mehrere Parameter der Zellreifung, einschließlich des Ruhepotenzials, des Eingangswiderstands, der Zellkapazität und der Membranzeitkonstante zeigten sich nach dem Schlaganfall zu reiferen Stadien verschoben. Die intrinsische Erregbarkeit war ebenso nach dem Schlaganfall erhöht und ein höherer Anteil der immaturen Neurone konnten schnelle Aktionspotenziale auslösen. Der morphologische Reifungsprozess der ABGCs entsprach jedoch der funktionellen Entwicklung nach dem Schlaganfall. Die exzitatorische synaptische Aktivität, gemessen als postsynaptische Spontan- oder Miniaturströme (s/mEPSC) war erhöht. Des Weiteren zeigten wir eine Entkopplung der intrinsischen und netzwerkbedingten Entwicklungsdynamik in postischämischen ABGCs mittels des allgemeinen linearen Modells.

Heraus resultieren junge, intrinsisch übererregbare Neurone, die unangemessen stark synaptisch erregt werden. Demzufolge weisen unsere Ergebnisse nicht nur auf einen beschleunigten Reifungsprozess der intrinsischen und synaptischen Erregbarkeit, sondern auch auf eine entkoppelte Entwicklungsdynamik hin, die ungünstige Effekte auf die Netzwerkfunktion und auf die Kognition haben kann. Die resultierende Übererregbarkeit der nach dem Schlaganfall entwickelten ABGCs kann zu Gedächtnisstörungen sowie zu einer erhöhten Anfälligkeit für epileptische Anfälle führen.

5 Introduction

5.1 Stroke and long-term neuropsychiatric symptoms

Stroke is one of the leading causes of death and disability in the developed world (Mukherjee and Patil, 2011). The acute treatment of stroke has seen some major advancements through both patient education (resulting a higher awareness of stroke-like symptoms and reducing the time to presentation), medical therapy (increasing the group of patients eligible for thrombolysis by expanding the thrombolysis time-window to 4,5 hours as well as imaging improvement aimed at better recognizing patients more likely to benefit from therapy) and interventional therapy (technical improvements of thrombectomy devices). These developments have resulted in a higher survival rate after stroke and better motor recovery of afflicted individuals. However, new challenges arise through the emergence of long-term neuropsychiatric complications of stroke, which may persist even up to 15 years after the ischemic insult (Teasdale and Engberg, 2005). Most frequent are not only epileptic seizures caused by structural changes, but also post-stroke depression and cognitive impairment. The pathophysiology of these increasingly prevalent syndromes is only little understood and presents a significant obstacle in improving the quality of life of stroke-afflicted individuals.

5.2 Post-stroke cognitive impairment and dementia

Cognitive impairment following stroke is often observed in clinical praxis. These cognitive deficits may persist and become chronic, influencing the quality of life and the rate of patients returning to work even in cases with only mild stroke (Mijajlovic et al., 2017;Henon et al., 2006). While motor deficits may show an improvement over time, cognitive deficits worsen progressively. The reason for this remains unknown, and further clinical clarification is limited due to general difficulties in study design in populations of stroke patients (Mijajlovic et al., 2017). In the traditional approach, one would compare the incidence of dementia in a population of stroke patients using a well-established test of cognitive function such as the Montreal cognitive assessment test (MoCA). This approach is however biased by the rate of cognitive decline previous to the stroke event and the aging-associated decrease in cognitive function (see Figure 1) and correcting for these confounding factors is challenging (Henon et al., 2006). Further studies are currently ongoing investigating serologic, genetic, immunologic and imaging markers of post-stroke cognitive impairment.

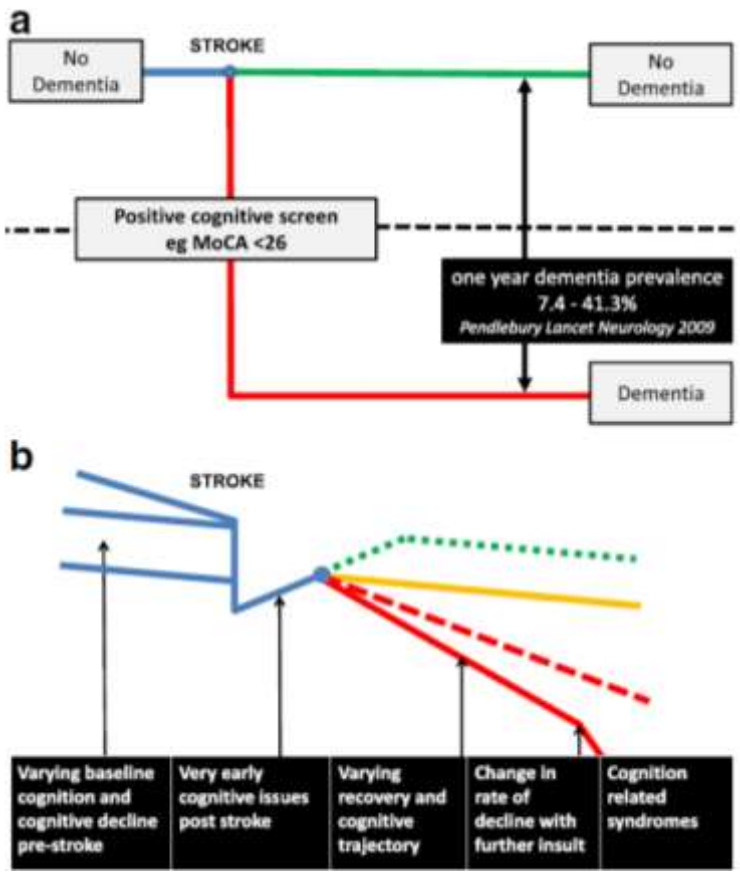


Figure 1: Two models of post-stroke cognitive impairment and dementia. a. In a simplified view, a subset of cognitively normal patients develop dementia after stroke. b. Real-life situations are more complex, with patients presenting with different levels of cognitive decline due to different underlying conditions and risk factors prior to stroke. After an acute worsening, there is a varying rate of cognitive recovery and a change in the rate of cognitive decline (modified from (Mijajlovic et al., 2017)

5.3 Post-stroke depression

Another common neuropsychiatric complication after stroke is depression, referred to as post-stroke depression (PSD). PSD is indeed considered the most common neuropsychiatric complication after an ischemic event (Robinson, 1997). Both major depression as well as minor depression may develop following stroke, with a peak after 3-6 months and a subsequent decline to about 50% one year after the insult. Its prevalence ranges from 20 to 60% (Robinson and Jorge, 2016). The development of PSD is associated with an increased mortality risk, even when compared to similarly impaired patients without PSD (OR 3.5, 95%CI = 1.4-8.4, p=0.007, (Morris et al., 1993)). Major and minor depression have different time courses. While major depression usually subsides within 1-2 years, it may also become chronic. The dynamic of minor depression is more variable, having both a short-term as well as a chronic disease course (Zavoreo et al., 2009). The pathophysiological mechanisms of PSD are still largely unknown, but adult neurogenesis is proposed to play a role (Loubinoux et al., 2012;Sahay et al., 2007).

5.4 Adult Neurogenesis

The discovery of adult neurogenesis by Altman (Altman, 1962;Altman and Das, 1965) changed one of the fundamental presets in neural sciences and uncovered a fundamentally new form of neuronal plasticity of the central nervous system. This was made possible by the newly developed method of [^{3}H]-thymidin autoradiography, which could label proliferating neurons. Using this method, Joseph Altman described proliferating neurons in the olfactory bulb and dentate gyrus of adult rats. However, research in the newly created field of adult neurogenesis did not gain traction until later studies confirmed the formation of new neurons in the adult mammalian brain, including in humans (Eriksson et al., 1998;Lois and Alvarez-Buylla, 1994;Doetsch, 2003). This was possible by the development of new synthetic thymidine analogues such as 5-bromide-3'-desoxyuridine (BrdU) which incorporates in new born neurons. In the last two decades, several studies addressed the identity of adult neural stem/precursor cells (NSPC), the stages undergone by developing neurons, the signaling pathways and regulating factors in health and disease, as well as the function of newly developed neurons (Piatti et al., 2013;Mu et al., 2010;Toda and Gage, 2018). There are two regions in the adult mammalian brain that harbor NSPC and where generation of new neurons persists throughout life: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Figure 2).

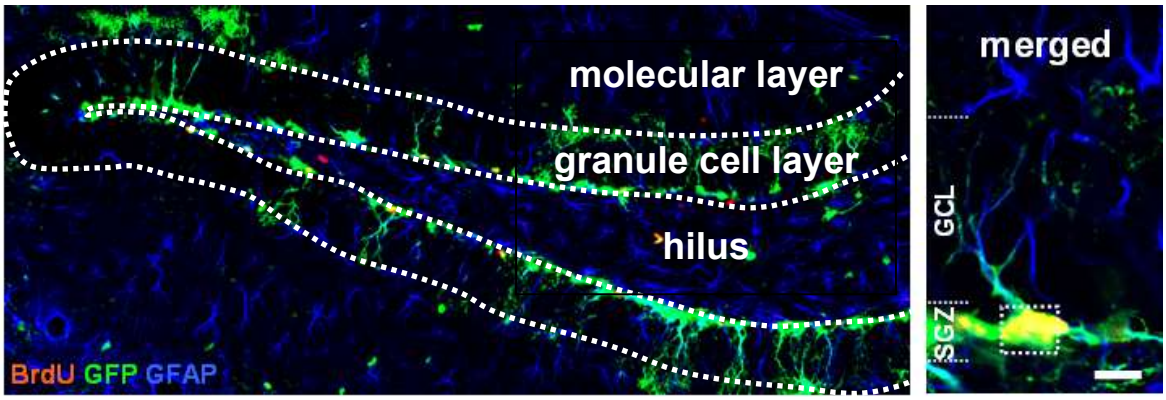
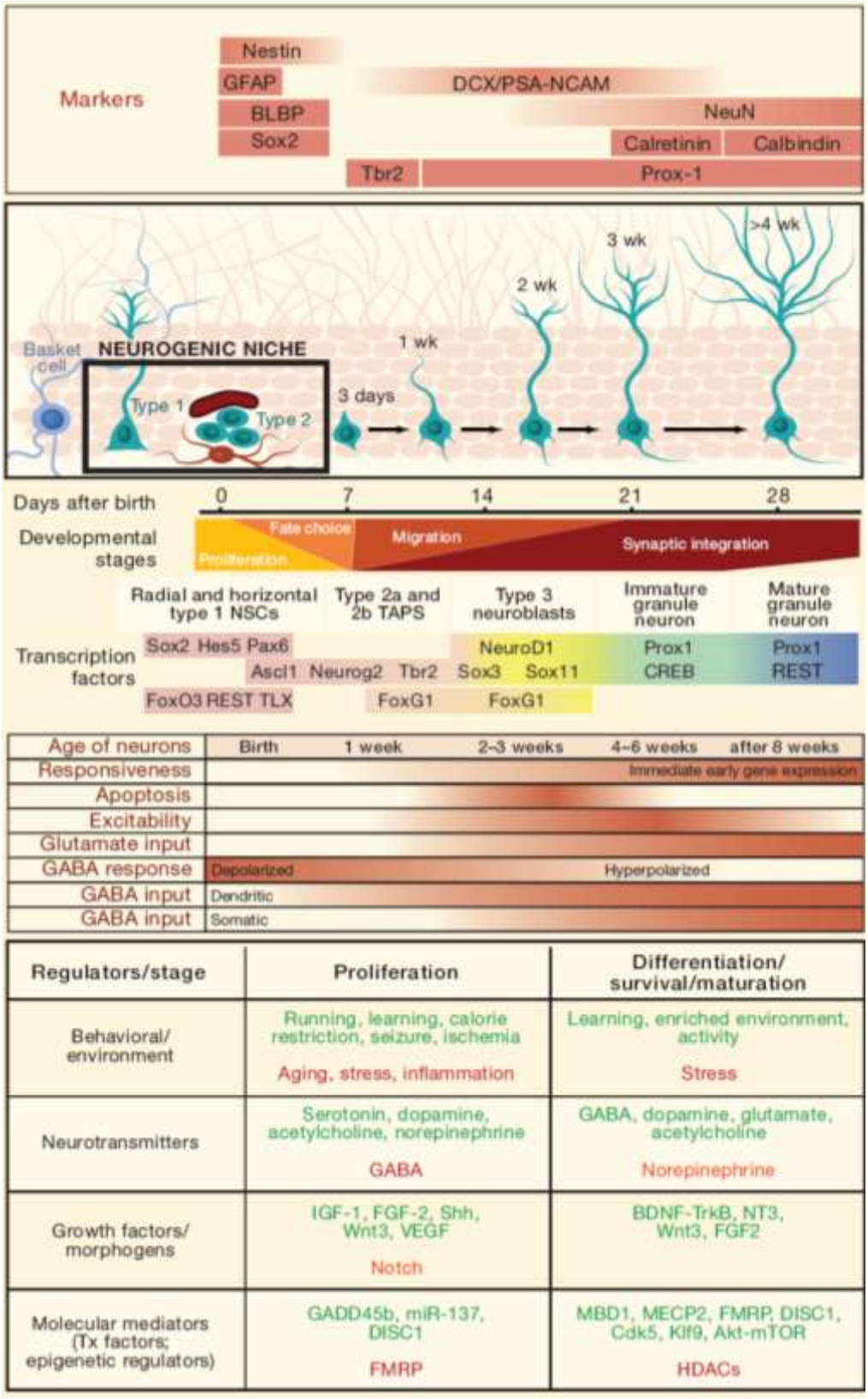


Figure 2: Neural stem/precursor cells (NSPC) in the subgranular zone of the hippocampal dentate gyrus. A subpopulation of astrocytes (blue, GFAP-positive) expresses green fluorescent protein (green, GFP) under the nestin-promoter, identifying these cells as neural progenitor cells. Intraperitoneally applied BrdU (red) is incorporated into dividing NSPC and can be used as a birth tracer to determine the age of the cells (right figure). (modified from (Keiner et al., 2010)

5.5 Neurogenesis in the dentate gyrus

In the adult dentate gyrus, new granule neurons develop continually from a local population of neural stem/precursor cells and integrate in the preexisting network (Figures 2,3), modulating its plasticity and function (Adlaf et al., 2017;Ikrar et al., 2013;Nakashiba et al., 2012;van Praag et al., 2002). The NSPC population is represented by glial fibrillary acidic protein (GFAP) positive radial glia-like cells (RGL, Type 1 cell). The cell bodies of RGL cells are located in the subgranular zone, which is the border between the granule cell layer and the hilus of the dentate gyrus (Figure 2). From there they extend a single apical process through the dentate cell layer and into the inner molecular layer. The RGL cells are a heterogenous population and have special characteristics which allow them to undergo both symmetrical and asymmetrical cellular division. Through symmetrical division, they give rise to two cells of similar geno- and phenotype, whereas by asymmetrical division the daughter cells have a different fate than the mother cell. Through symmetrical division NSPC show their capacity for self-renewal, while by asymmetrical division they contribute new neurons to the dentate network. In the physiological state, RGL are relatively quiescent and divide asymmetrically to give rise to a transiently amplifying group of neuroblasts, of which only a small percentage survives to differentiate to mature neurons (Figure 3). During this differentiation process, cells go through different stages (Type 2a, 2b, and 3) characterized by different protein expression patterns, regulatory checkpoints, morphology and intrinsic excitability properties, before developing into functional granule cells (Toda and Gage, 2018). The neuronal phenotype appears about 7-10 days postdivision, and the maturation stages of neuronal precursor cells in the adult brain recapitulate those during embryogenesis. Cells initially sprout an axon towards the CA3 region of the hippocampus, before dendrites start reaching through the granule cell layer towards the molecular layer. At this early stage, immature neurons may have a bipolar morphology by presenting a basal dendrite (Lubbers and Frotscher, 1988;Rao and Shetty, 2004), although they later retain a single apical dendrite. Presence of dendritic spines at around 14 days postdivision shows early integration in the preexisting neuronal network at this stage. This integration progresses up to and beyond 28 days postdivision, as neurons acquire a mature morphological and electrophysiological phenotype. The maturation process is carefully orchestrated as cellular migration, intrinsic excitability, network integration and output organization evolve in a specific pattern and are dynamically interlinked (Figures 3, 4). Initially, neuroblasts have a depolarized membrane potential at around -40 mV and a very high input resistance (over 1GΩ and up to 10GΩ).



Regulators/stage	Proliferation	Differentiation/ survival/maturation
Behavioral/ environment	Running, learning, calorie restriction, seizure, ischemia Aging, stress, inflammation	Learning, enriched environment, activity Stress
Neurotransmitters	Serotonin, dopamine, acetylcholine, norepinephrine GABA	GABA, dopamine, glutamate, acetylcholine Norepinephrine
Growth factors/ morphogens	IGF-1, FGF-2, Shh, Wnt3, VEGF Notch	BDNF-TrkB, NT3, Wnt3, FGF2
Molecular mediators (Tx factors; epigenetic regulators)	GADD45b, miR-137, DISC1 FMRP	MBD1, MECP2, FMRP, DISC1, Cdk5, Klf9, Akt-mTOR HDACs

Figure 3: Overview of the process of adult neurogenesis in the hippocampal dentate gyrus highlighting protein markers of different developmental stages, transcription factors and regulators of proliferation and differentiation. Modified from (Vadodaria and Gage, 2014)

The intracellular chloride concentration ($[Cl]_i$) is increased due to a high ratio of NKCC1/KCC2, driving the reversal potential of Cl^- towards depolarized values. Consequently, GABA acting through $GABA_A$ receptor channels depolarizes the cells, leading to calcium influx and/or facilitating generation of action potentials (Heigele et al., 2016). Such immature neurons can produce non-overshooting, single broad spikelets driven by calcium influx (Schmidt-Hieber et al., 2004). At this early stage, glutamatergic input is not yet present, while GABAergic input is mainly perisomatic and excitatory/depolarizing in effect. During further maturation, the expression ratio of NKCC1/KCC2 decreases, and consequently $[Cl]_i$ decreases as well, lowering the chloride reversal potential below the resting potential of the neuron. This leads to GABA inducing hyperpolarizing responses through $GABA_A$ receptor channels. At around three weeks postdivision, these newborn neurons begin formation of glutamatergic synapses. During this time young neurons regulate their intrinsic and synaptic excitability in concert. Electrotonic properties such as input resistance and membrane time constant decrease, action potentials become faster and larger, output gain decreases, and synaptic excitation increases (Schmidt-Salzmann et al., 2014). All these changes counterbalance each other, with the net effect of stabilizing neuronal function. Even so, the functional properties are, however, still different from older neurons as new neurons demonstrate enhanced long-term potentiation (LTP) (Schmidt-Hieber et al., 2004). New neurons will become electrophysiologically and morphologically indistinguishable from fetal-born granule cells within about 20 weeks in the rodent brain. This developing time course of adult-born granule cells (ABGCs) is prolonged in higher mammals and may take over 6 months to complete in humans (Kohler et al., 2011).

5.6 Regulators of hippocampal adult neurogenesis

The process of adult hippocampal neurogenesis is regulated by many factors at different levels, ranging from behavioral to local and genetic influences, which can affect both the proliferation rate of NSPC and the differentiation and survival rate of new neurons (see Table 1). The neurogenic niche in the dentate gyrus constitutes a particular microenvironment, which supports self-renewal and differentiation on NSPC (Doetsch, 2003). Important factors of the neurogenic niche are represented by the vasculature, the extracellular matrix and the basal lamina, as well as the local population of glia cells, including astrocytes and microglia which secrete cytokines and chemokines. Furthermore, astrocytes and microglia cells also control the synaptic integration and function of ABGCs in the dentate gyrus

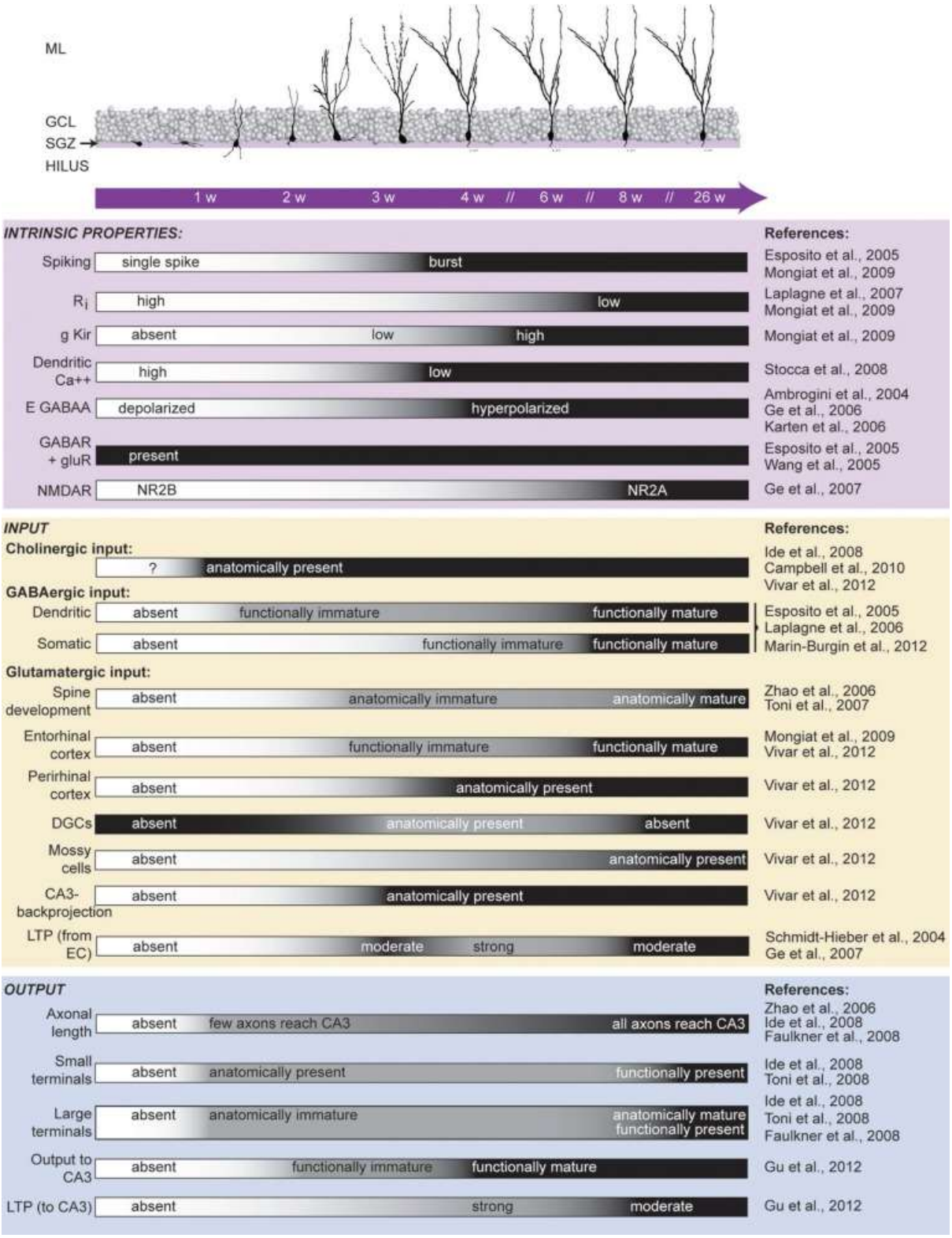


Figure 4: Overview of the process of adult neurogenesis in the hippocampal dentate gyrus highlighting the maturation of intrinsic excitability and network integration (from (Piatti et al., 2011)

(Sultan et al., 2015;Beckervordersandforth et al., 2017). Extrinsic and environmental factors also modulate adult hippocampal neurogenesis. Indeed, enriched environment and running as well as specific diet have become standard paradigms to increase the rate of neurogenesis and promote neuronal survival of ABGCs (van Praag 1999, Kempermann 1997). On the other hand, inflammation, stress and aging detrimentally influence adult neurogenesis. Both extrinsic and intrinsic regulators act through a plethora of biochemical factors, as listed in Table 1. Importantly, local network activity also regulates adult neurogenesis, which is than itself shaped by the function of new neurons. Just as during embryonic development, GABA plays an important role in the differentiation and maturation of new neurons, being necessary for dendritic arborization and synapse formation. Another important factor is BDNF, which is released from the underlying substrate hippocampal neurons in response to synaptic activity and is required for activity-dependent stem cell differentiation (Babu et al., 2009).

5.7 Functional Role of Adult-Born Hippocampal Neurons

The function of adult neurogenesis has been investigated using different strategies to ablate neuronal proliferation and examining cognitive performance, including learning and memory. The unique plasticity rules of newly generated granule cells include a limited time window of hyperexcitability and enhanced plasticity during the first month postdivision. Young ABGCs thus contrast mature granule cells, which are hyperpolarized, relatively silent, and thought of as gating the input into the hippocampal network. ABGCs are considered to be centrally involved in the formation of spatial memory. Particularly, they are required for the differentiation of complex spatial patterns challenging the hippocampal function through a process known as pattern separation (Sahay et al., 2011). Hence, they allow a greater precision of spatial navigation in complex environments. Old, resident neurons, on the other hand, are thought to mediate the process of pattern completion. Different strategies have been used to manipulate adult neurogenesis in order to study the functional outcomes, including chemically ablating adult neural stem cells by e.g. methylazoxymethanol acetate (MAM) or temozolomide (TMZ), but also physical methods such as X-ray irradiation and genetic ablation in transgenic animals (Zhao et al., 2008). Further, differentiating between hippocampus-dependent and hippocampus-independent learning, as well as neurogenesis-dependent memory tasks has required new experimental assays. The gold standard for testing hippocampal function is the Morris water maze (MWM), which has been modified to comprise complex visual landmarks and goal reversal at day 4, which particularly challenges

Proliferation	
miR-124	Decreases proliferation
Shh	Increases proliferation
Sox2	Increases proliferation
Tlx	Increases proliferation
Wnt	Increases proliferation
Differentiation	
Transcription factors	
AscH	Overexpression generates oligodendrocytes
	Expressed in NSCs to produce GABAergic interneurons in OB
	Expressed in NSCs to produce glutamatergic neurons in hippocampus
Neurog2	Expressed in NSCs to produce glutamatergic neurons in hippocampus
	Expressed in NSCs to produce glutamatergic neurons in svz
Tbr2	Expressed in NSCs to produce glutamatergic neurons in svz
Epigenetic mechanisms	
Gadd45b	Necessary for dendritic arborization
MBD1	Necessary for neuronal differentiation
MeCP2	Necessary for neuronal maturation
MII1	Necessary for neuronal differentiation in svz
Migration	
IGF-1	Necessary for neuroblast migration
Shh	Necessary for neuroblast migration
Integration	
Extrinsic factors	
BDNF	Increases neuronal survival and dendritic arborization
FGF-2	Necessary for synaptic plasticity
GABA	Necessary for dendritic arborization and synapse formation
Glutamate	Necessary for neuronal survival and synaptic plasticity
NT-3	Necessary for synaptic plasticity
Intrinsic factors	
Cdk	Necessary for neuronal survival and dendritic arborization
CREB	Increases neuronal survival and dendritic arborization
DISC1	Decreases synaptic integration
Klf-9	Increases synaptic integration
NeuroDI	Necessary for neuronal survival and maturation

Table1: Biochemical modulators of adult neurogenesis (modified after (Mu et al., 2010))

the hippocampal function. Separating similar but distinct complex visual patterns is improved by new neurons, which allows rapid adaptation to a new context (Garthe et al., 2009). Further studies also found a role for adult neurogenesis in modulating anxiety-like behavior and being a target of antidepressant treatment (Encinas et al., 2006;Sahay and Hen, 2007;Sahay et al., 2011).

5.8 Hippocampal neurogenesis following stroke

Pathological conditions such as epilepsy, trauma, inflammation and stroke affect the process of adult neurogenesis in the hippocampus in different ways (Chugh et al., 2013;Jakubs et al., 2006;Villasana et al., 2015;Jakubs et al., 2008;Kluska et al., 2005;Liu et al., 1998). Stroke strongly and robustly increases the proliferation and differentiation of NSPC in the dentate gyrus, which has been thought to be a beneficial compensatory mechanism. This process is proportional to the infarct volume and takes place even as the ischemic lesion is remote from the hippocampus. Hence, small cortical infarcts, e.g. as induced by photothrombosis, produce only a modest increase in dentate neurogenesis as compared to larger, cortico-subcortical infarcts due to medial cerebral artery occlusion (Niv et al., 2012). These postischemic ABGCs are functionally recruited by the hippocampus network during cognitive tasks as evidenced by the expression of immediate early genes. Surprisingly, and in contrast to the standing hypothesis, the higher number of granule cells does not correlate with better functional outcomes (Geibig et al., 2012). Furthermore, increasing postischemic neurogenesis by running does not result in better cognitive abilities but seems rather to aggravate hippocampal-dependent memory deficits (Woitke et al., 2017). A possible explanation may be a higher incidence of abnormalities in the migration and morphology of these cells despite the increased number of ABGC following stroke (Figure 5) (Niv et al., 2012). The question therefore arises if the enhanced hippocampal neurogenesis following brain infarcts is maladaptive and has a deleterious effect on cognitive function. So far, a direct electrophysiological characterization of ABGCs after stroke is lacking. Therefore, it is not known if the maturation and integration processes develop normally. This would also clarify the important question if deleterious input to the dentate gyrus is underlying hippocampal malfunction or if the new neurons themselves are dysfunctional. Pathological changes in their excitability and network integration may lead to secondary defects following stroke such as neurocognitive dysfunction and higher seizure susceptibility.

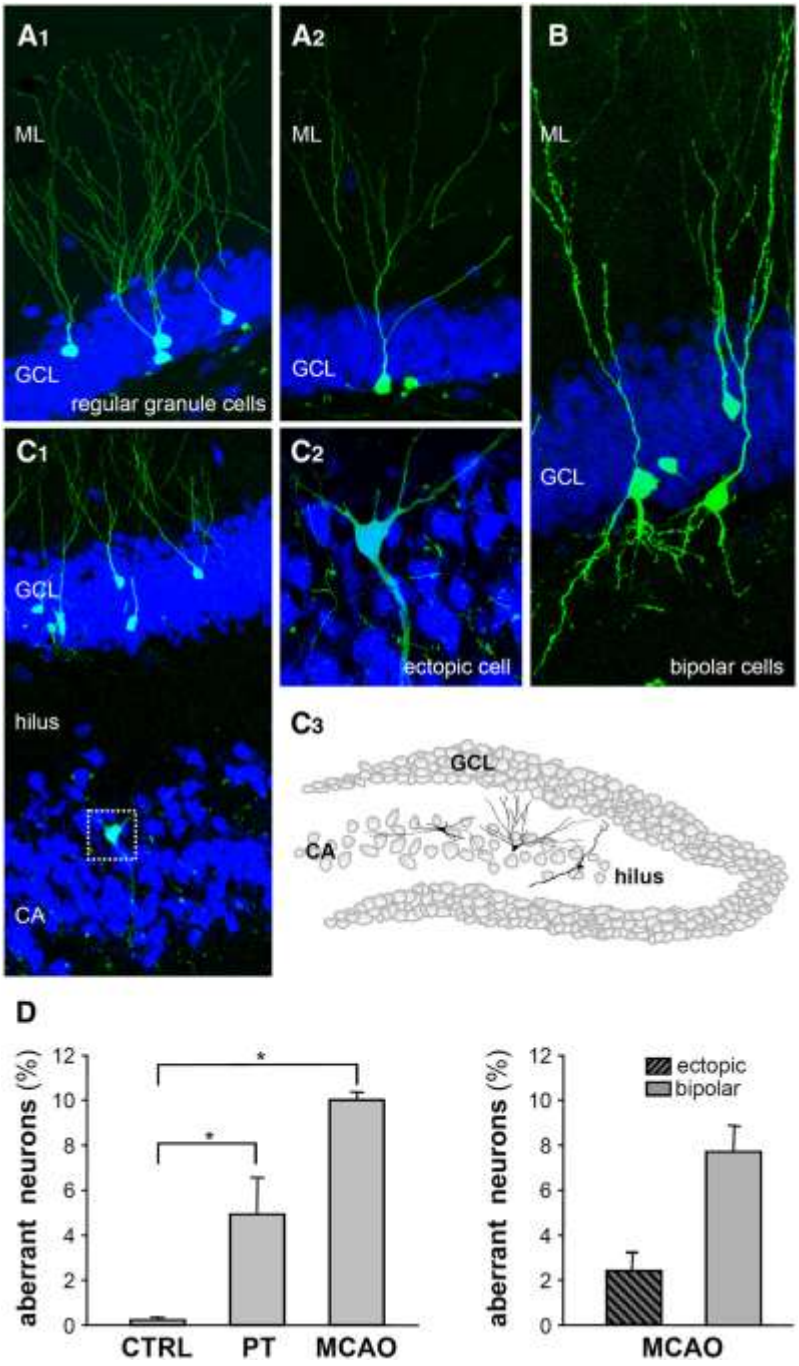


Figure 5: Morphology of ABGCs after stroke. ABGCs are retrovirally labeled with the green fluorescent protein (GFP). Normal gross morphology is shown in A, while examples of pathological differentiation and migration are shown in B and C (bipolar morphology and ectopic cells, respectively). The extent of postischemic aberrant neurogenesis correlates with infarct size (D). GFP, green fluorescent protein; GCL, granule cell layer; MCAO, middle cerebral artery occlusion; ML, molecular layer; NeuN, neuronal nuclei; PT, photothrombotic infarcts. Modified from (Niv et al., 2012).

6 Scope

Stroke has been repeatedly shown to robustly stimulate adult neurogenesis in the dentate gyrus of the hippocampus, but the functional consequences of this increase in newborn neurons remained uncertain. While under physiological conditions the generally assumed hypothesis that more granule cells equate with improved cognition may hold true (Sahay et al., 2011), this may not be the case or even reversed following brain damage. Two previous studies showed morphological and behavioral results suggesting maladaptive role for ABGCs after a stroke. While aberrant migration and morphological differentiation may play a role in network dysfunction through generation of ectopic or bipolar neurons, such aberrant cells represent only a minority of postischemic ABGCs. So far, the function of morphological normally appearing postischemic ABGCs as well as the integration in the preexisting network has not been investigated to date.

Therefore, the scope of this project was to characterize the electrophysiological properties of intrinsic and network excitability in ABGCs two weeks following an ischemic event. The well-established MCAO model of cerebral ischemia was chosen based on previous results showing extensive cortical and subcortical infarcts that do not, however, affect the hippocampus directly, but that lead to a higher rate of ectopic migration and aberrant morphology. Recorded cells were also filled with biocytin, enabling subsequent morphological reconstruction. Birth tracing by intraperitoneal application of BrdU was done to monitor the expression pattern of DCX. Mature, DCX negative (DCX-) granule cells were also recorded to provide a physiological control for normal maturation parameters.

7 Original publication

Stroke Accelerates and Uncouples Intrinsic and Synaptic Excitability Maturation of Mouse Hippocampal DCX+ Adult-Born Granule Cells.

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Suggested cover art

Neurobiology of Disease

Stroke Accelerates and Uncouples Intrinsic and Synaptic Excitability Maturation of Mouse Hippocampal DCX$^+$ Adult-Born Granule Cells

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Stroke robustly stimulates adult neurogenesis in the hippocampal dentate gyrus. It is currently unknown whether this process induces beneficial or maladaptive effects, but morphological and behavioral studies have reported aberrant neurogenesis and impaired hippocampal-dependent memory following stroke. However, the intrinsic function and network incorporation of adult-born granule cells (ABGCs) after ischemia is unclear. Using patch-clamp electrophysiology, we evaluated doublecortin-positive (DCX$^+$) ABGCs as well as DCX$^-$ dentate gyrus granule cells 2 weeks after a stroke or sham operation in DCX/DsRed transgenic mice of either sex. The developmental status, intrinsic excitability, and synaptic excitability of ABGCs were accelerated following stroke, while dendritic morphology was not aberrant. Regression analysis revealed uncoupled development of intrinsic and network excitability, resulting in young, intrinsically hyperexcitable ABGCs receiving disproportionately large glutamatergic inputs. This aberrant functional maturation in the subgroup of ABGCs in the hippocampus may contribute to defective hippocampal function and increased seizure susceptibility following stroke.

Key words: aberrant integration; adult neurogenesis; doublecortin; hyperexcitability; stroke; uncoupled maturation

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9 Discussions

The discovery of adult neurogenesis has changed the presets of brain plasticity and added a new and dynamic field of research. It also raised multiple questions regarding the role of neurons born in the adult brain, the mechanisms responsible for regulating the processes of proliferation, differentiation and maturation, but also about the pathology associated with a disturbance in this well-balanced system, either from intrinsic causes or by systemic or brain diseases. It has been shown numerous times that a pathological environment causes a modification in the normal dynamics of adult neurogenesis, but the functional consequences of these changes and whether they are beneficial or maladaptive have still being disputed and are the focus of many research studies. The systemic effects that are possibly induced by newly generated neurons is itself an active area of research, as there exist only few experimental paradigms allowing a fine dissection of hippocampus-dependent and of neurogenesis-dependent processes. Currently, best evidence is available for a modified version of the Morris water maze which is thought to reflect changes in neurogenesis related hippocampal learning. Behavioral, lesional and computational studies suggest a role in hippocampus-dependent memory functions such as learning, memory and cognitive plasticity, but also in pattern separation (a computational process used to discriminate between two similar but different inputs) and emotion (anxiety and depression) (Toda and Gage, 2018).

Cerebral ischemia has been shown to induce a robust increase in hippocampal neurogenesis through remote mechanisms. This led to the hypothesis that the changes are adaptive in nature, and hence more granule neurons are beneficial for the brain. This seems plausible in a physiological state, where higher rates of neurogenesis and increased number of cells in the dentate gyrus may improve cognition (Sahay et al., 2011), but the same may not necessarily be true under pathological conditions. Despite evidence of neuronal activation of postischemic ABGCs and despite the higher number of cells present in the hippocampus following stroke, no evidence of improved cognition were found (Geibig et al., 2012). When further increasing the rate of adult neurogenesis after stroke by running , mice also fail to show an improvement in hippocampal-dependent memory function and event point towards a worsening of memory deficits (Woitke et al., 2017). Aberrant morphological maturation and ectopically migrated neurons provided a possible explanation, still, the overall rate of such

gross morphological abnormalities remained relatively small and most ABGCs showed normal morphology and integrated in the preexisting dentate network (Niv et al., 2012). Based on these previous observations from our laboratory, we further addressed the important questions of whether the newly generated neurons are functional and normally integrated in the hippocampal network. We found that the processes of intrinsic maturation and network integration of DCX+ ABGCs is tightly coupled under physiological conditions and functions to counterbalance a high intrinsic excitability through a weak synaptic activation. In contrast to the physiological maturation dynamics, we found that stroke strongly accelerates both, the generation of mature action potentials in postischemic ABGCs, as well as passive, electrotonic cellular properties. Intrinsic excitability, however, as expressed by output gain, does not follow this time course and remains at a level of hyperexcitability characteristic of immature neurons. Finally, enhanced synaptic excitation further tips the balance towards hyperexcitability. In conclusion, the well balanced and coupled process of maturation of intrinsic and synaptic excitability is completely disturbed in the pathological condition after stroke. These effects were specific for DCX+ cells, since DCX- neurons were differently affected by stroke.

While we did not explore in our study the mechanisms responsible for these differences in maturation after stroke, possible effectors are the many neurotransmitters, cytokines and growth factors released during the ischemic event (Hazell, 2007). The remote effect of stroke on hippocampal adult neurogenesis, the size of the effect being proportional to the infarct volume and the volume dispersion of the effect (ipsilateral hippocampus is more affected than the contralateral one) are possible hints towards a paracrine effect of stroke. Furthermore, BDNF, an important regulator of ABGCs survival and differentiation, exhibits a concentration peak in the cerebrospinal fluid after stroke, which was found to promote neuronal stem cell proliferation, migration and differentiation after subarachnoid hemorrhage (Hjalmarsson et al., 2014;Lee et al., 2016).

Several other pathological stimuli besides stroke can result in a disruption of adult neurogenesis in the hippocampus. The underlying pathological event and the degree of damage (e.g. after epileptic, ischemic, traumatic, or inflammatory lesions) seem to be the determinants of the degree to which adult neurogenesis is affected. Interestingly, cellular maturation and functional integration after a lesion seem to develop in a pathology-specific

manner. For example, profound morphological changes after experimental traumatic brain injury do not preclude physiological maturation and integration of ABGCs under these conditions (Villasana et al., 2015). After status epilepticus different induction protocols can result in different outcomes regarding hippocampal adult neurogenesis. After local electrical stimulation, ABGCs showed reduced network excitatory drive, which is caused presynaptically by a reduced release probability (Jakubs et al., 2006). In a model of pilocarpine induced status epilepticus, immature neurons developed an accelerated intrinsic maturation and premature development of excitatory synaptic drive, similar to our findings. As these changes also include recurrent polysynaptic inputs (Overstreet-Wadiche et al., 2006;Villasana et al., 2015), the local environment in which ABGCs mature is fundamental to the physiological development of new cells. Pathological local environments may drive different responses regarding their maturation and integration (Wood et al., 2011). It is therefore plausible, that a compensatory, beneficial effect or a detrimental, maladaptive effect of new neurons born in a pathological environment may also be condition-specific (Hester and Danzer, 2013;Hosford et al., 2016;Surget et al., 2011).

10 Conclusion

In conclusion, our results provide evidence that immature, morphologically normal DCX+ ABGCs undergo an accelerated functional maturation following stroke. They develop fast rising action potentials prematurely, resembling those of mature granule cells, while still having an increased input resistance and hence an increased responsiveness to incoming excitatory currents. Their output gain is increased in both instantaneous frequency and in number of action potentials, a property characteristic of immature neurons. Furthermore, they receive greater synaptic excitation, probably mediated by postsynaptic glutamate receptor changes. The dynamics of intrinsic and synaptic maturation are however uncoupled as compared to ABGCs undergoing physiological maturation. These mechanism result in new neurons with unbalanced excitation through a synaptic hyperexcitability potentiating an increased intrinsic excitability. Thus, normal neuronal morphology does not preclude aberrant cellular function and pathological network integration. These results complement current morphological and behavioral data on the consequences of stroke on the maturation and integration of ABGCs in the adult hippocampus. We uncover details of functional dysregulation of postischemic ABGCs and provide a putative mechanism which could contribute to persistent cognitive disabilities in stroke patients.

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12 Appendix

12.1 Index of figures

12.2 Index of tables

12.3 Curriculum vitae

Mihai Ceangă

Born on 4th November 1984 in Galaţi, Romania

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PROFESIONAL EXPERIENCE

June 2015 – Present	Resident physician, Hans-Berger Department of Neurology, Friedrich-Schiller University, Jena
April 2014 – March 2015	Resident physician, Department of General Neurology, Westphalian Wilhelms-University of Münster
Jan. 2012 – Feb. 2014	Resident physician, Hans-Berger Department of Neurology, Friedrich-Schiller University, Jena
June 2010 – Dec. 2011	Resident physician, Department of Neurology, Medical Centre Chemnitz

EDUCATION

2003 – 2009	General medicine, Bachelor of medicine and surgery, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
2007 – 2008	Erasmus scholarship at the "Lyon Nord" Faculty of Medicine, Lyon, France
1999 – 2003	„Vasile Alecsandri" High School, Galati, Romania

RESEARCH EXPERIENCE

2016 – 2017	IZKF scholarship on "Impact of human monoclonal anti-NR1-antibodies of the synaptic plasticity and network activity in a mouse model of NMDAR encephalitis". (Workgroup Prof. Dr. med. C. Geis).
2012 – 2013	IZKF scholarship on "Aberrant neurogenesis after experimental stroke". (Workgroup Prof. Dr. med. Ch. Redecker).
2004 – 2008	Research assistant in the neuroscience laboratory of Prof. L. Zagrean (Workgroup of Dr. A.-M. Zagrean) on the projects: - Research Project CEEX M3 no. 242/2006, The Development of a European Network for the Investigation of Interneuronal

and Neuroglial Relations in Energetic Stress, Neurodegenerative Diseases and Ageing, Aiming the Development of New Therapeutic Strategies, supported by the Ministry of Education, Research and Technology of the Romanian Government, 2006 – 2008.

- Research Project Viasan no. 253, Cellular and Molecular Study of the Effects of Hypoxia on Neuronal Development and Excitability in vitro, supported by the Ministry of Education, Research and Technology of the Romanian Government, 2003 – 2005.

TEACHING EXPERIENCE

2016 – Present Neurology tutor at the Hans-Berger Department of Neurology

LANGUAGES

English (CAE, TOEFL iBT), French, Romanian, German – fluent.

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Finally, I want to thank my wonderful wife Andreea Ceangă for her love, patience and support and also my parents, Emil Ceangă and Rodica Ceangă, who have constantly supported me throughout my education.

12.5 Ehrenwörtliche Erklärung

Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich- Schiller-Universität bekannt ist,

ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben:

Prof. Dr. med. Christoph Redecker
Dr. med. Albrecht Kunze
Prof. Dr. med. Christian Geis

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen,

dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und

dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Ort, Datum — Unterschrift des Verfassers

Jena, 15.10.2019